Microbial Ecology of Polyacrylamide Application in Agricultural Soils

by Jeanine L. Kay-Shoemake and Mary E. Watwood

High molecular weight water soluble polyacrylamides (PAMs) are currently being added to irrigation water to control soil erosion associated with flood-furrow and sprinkler irrigation. Applied at 10 mg/L to irrigation water early in the set (0.7- 3.0 Kg / ha), PAM has been shown to reduce furrow sediment loss in Idaho by an average of 94%; physical binding of PAM to soil results in stabilization of soil aggregates (15). Water soluble PAMs have been authorized, or authorization is expected, for agricultural use in many western states in the next several years. It is estimated that 2 million irrigated acres in the northwestern United States, and 300 million irrigated acres worldwide, are suitable for treatment. In the near term, it is likely that large amounts of PAM will be applied annually to large areas of irrigated farm land.

The type of PAM found to be most effective in reducing erosion has a very high molecular weight (10-20 x 10^6 MW), and a linear, anionic configuration. It is a copolymer made up of approximately 82 mole % acrylamide subunits and 18 mole % acrylate subunits (14). The resulting polymer consists of repeating ethylene units in the backbone, with amide

and carboxylic acid substituents as side chains. This PAM exists as an anionic species in an environment of pH 6 or above; the anionic characteristic is responsible for the soil stabilizing effect, via complex electrostatic interactions with soil aggregates.

Substantial research has focused on PAM's ability to reduce soil erosion via physical-chemical interaction with soil aggregates (for review see ref. 15). However, little is known about microbial degradation of the polymer, the impact of PAM on the microbial ecology of agricultural soils, or potential effects of degradation products on the system. There are contradictory statements in the available literature regarding the biodegradation of PAM. Azzam et al. (3) state that physical degradation of high molecular weight PAM is approximately 10% per year and that little or no microbial degradation occurs, although no data is available to support this. It has also been reported in a lay agricultural publication that PAM degrades into CO_2, NH_3, and water (21), again with no data provided in support of this statement. Several PAM biodegradation studies have been conducted anaerobically to determine effects of PAM degradation in flooding solutions used for enhanced oil recovery (8). Anaerobic conditions are not found typically in the surface soils of agricultural systems, so these results have limited applicability. There are several reports citing increased growth response of aerobes grown in the presence of PAM (7, 17), however, the cause of this effect is not known.

The biodegradability of PAM by soil microbes in an agricultural setting is yet unknown and the impact of PAM application on microbial communities and nutrient cycling is unclear. The objectives of this study were to determine:

1) the impact of PAM additions on soil bacterial numbers.

2) the potential for PAM biotransformation as a carbon or nitrogen source,

3) possible correlation between PAM treatment and changes in soil inorganic nitrogen pools, and

4) possible mechanisms of microbial utilization.

Material and Methods — Study Area

The USDA Agriculture Research Service Site in Kimberly, Idaho, is one of the leading research sites testing the erosion control effectiveness of PAM. This research has been ongoing at the site since 1991. The PAM study site consists of a total area of 2 ha (5 ac) divided into discrete experimental plots. The soil is Portneuf silt loam (coarse-silty, mixed, mesic, Durixerollic Calciorthid), with a pH of approximately 7.8 and a 2-8% $CaCO_3$ equivalent in the top soil.

The two experiments in progress at the time of field sampling were identified as the 'long-term' and 'managed-inflow' studies. The field layout is a randomized block design with three replications. Treatments in the 'managed inflow' study consist of a normal-inflow PAM (22.6 L/min), a high-inflow PAM (45.1 L/min) and their respective controls. The field was planted to potatoes (var. Russet Burbank). The 'long-term' study includes three treatments: control, PAM and Oil-PAM emulsion. The plot was planted to dry pink beans ('Viva Pink' *Phaseolus vulgaris L.*). The potato field received N fertilizer (80 lb/ac) as a urea-ammonium-nitrate mix (solution 32); the bean fields were not treated with chemical fertilizer.

In both studies, PAM-treated irrigation water contained 10 ppm PAM during the initial advance, followed by delivery of untreated water for the remainder of the irrigation set. This treatment has been ongoing since 1992 in the 'long term' plot. During the growing season the potato field was irrigated twice a week, while the beans were irrigated once every 10-14 days.

Soil Sample Collection

Approximately 100 gm of soil was collected from the upper 3 cm of each sampled furrow bottom, at a distance of 0.5 m, 1.0 m and 1.5 m from the irrigation inlet. Samples were combined, sieved through a 4 mm screen, and stored for no more than 2 weeks at 4+C. Composite samples were obtained for each crop type and each treatment type, in triplicate. Field samples were analyzed directly and also served as inoculum for enrichment cultures. Chemicals Polyacrylamide (PAM) identified as E-4103, used in enrichment cultures, was provided by Cytec Industries, Stamford CT, had a molecular weight of 1-2 $x10^7$, 18 mole % anionic charge, and was free of N containing contaminants. The polymer is comparable to the commercially available magnifloc 836A described in more detail by Lentz et al. (14) which was the formulation used in the field applications. In addition, Cytec Industries provided PAM-like molecules of reduced MW for use in enrichment cultures: E-4101 (200,000 MW), E-4100 (12,000 -15,000 MW), and E-4099 (3,000 - 4,000 MW). Acrylic acid (AA; 99% pure), formamide (reagent grade), propionamide (97% pure) and methylsulfonic acid (MSA; 99% pure) were obtained from Aldrich Chemicals (Milwaukee, WI). Acrylamide (AM; > 99% pure), and all other chemicals (reagent grade) were purchased from Sigma Chemical (St. Louis).

Enumeration of Bacteria in Soils

Microbial populations in soil samples were enumerated by the heterotrophic plate count method on soil extract agar and by the acridine orange direct count (AODC) method. Soil samples used in the heterotrophic plate counts were diluted in sterile 0.85% NaCl aqueous solution, and the various dilutions were plated in 0.1 mL quantities on to soil extract medium that was prepared as described by Wollum (25).

AODC analysis was performed on soil samples by the method described by Schmidt and Paul (20).

Inorganic Nitrogen Pool Determination

Soil samples (10 g) were extracted with 100 mL of 2 M KCl by the method described by Keeney and Nelson (12), and then refrigerated for no longer than 1 week prior to analysis. Nitrate-N concentration in each extract was determined by UV spectroscopy (Gilford, model 2600) at 210 nm and 301 nm, as described by Keeney and Nelson (12). Ammonia-

N in the extracts was determined by an ammonia specific electrode (Orion, model 95-12).

Enrichment Cultures and Growth Curves

Soil samples (0.5 g) from PAM treated and untreated furrows were inoculated into 25 mL of a mineral salts medium consisting of KH_2PO_4 (0.94 g), $Na_2HPO_4 \cdot 7H_2O$ (17.74 g), $CaCl_2$ (14.7 mg), $MgSO_4 \cdot 7H_2O$ (0.24 g), and 1 mL of a trace metals solution (see below) per L, at a pH of 7.6. PAM, AM, or AA was added to separate batches of media to investigate whether enrichment populations could be derived with the ability to utilize these substrates as a sole carbon or nitrogen source. AM, AA or PAM was added to give a final concentration of 0.05% in the medium. When AM, AA, or PAM was to be used as the sole C source, the medium was supplemented with 0.2% NH_4NO_3. When AM or PAM was to be used as the sole N source, media was supplemented to contain 0.1% glucose, or 0.05% acetate and 0.05% mannitol. The trace metals solution consisted of H_3BO_3 (2.85 g), $MnCl_2 \cdot 4H_2O$ (1.8 g), $FeSO_4 \cdot 7H_2O$ (1.35 g), $CoCl_2 \cdot 6H_2O$ (0.04 g), $CuCl_2 \cdot 2H_2O$ (0.03 g), $Na_2MoO_4 \cdot 2H_2O$ (0.03 g) and $ZnCl_2$ (0.02 g), in 1.0 L of distilled water. Controls consisted of appropriately supplemented mineral salts medium and inoculum, but without PAM, AM or AA added.

The cultures were incubated at 30°C on a reciprocal shaker (200 rpm). An aliquot of the culture was transferred to fresh medium every five days, for a total of seven transfers.

Growth curve experiments were conducted on each of the enrichment cultures exhibiting visible growth. Culture was added to mineral salts medium supplemented to contain the same components on which the culture had been established. The cultures were incubated at 30°C on a reciprocal shaker and absorbance of the cultures at 520 nm was determined at regular time intervals.

Abiotic Release of NH_3/NH_4^+ in Culture

Mineral medium (50 mL) supplemented with PAM, mannitol, and acetate (described above except no bacterial inoculum was added) was dispensed into a 500 mL Erlenmeyer flask and placed onto a reciprocal shaker at 30° C for 72 hours. Samples were removed at 24 hour intervals, and then analyzed for NH_4^+-N via ion chromatography. Ammonium was quantified by ion chromatography using a Dionex 100, equipped with a CS12 column for cation detection, cation self regenerating suppressor and a conductivity detector. Twenty mM methylsulfonic acid (MSA) was the eluent used at a flow rate of 1.0 mL/min.

UV Exposed PAM and SEHPLC

The bonds that make up the backbone of PAM are vulnerable to cleavage through exposure to UV irradiation and shear forces (18, 24). In the soil environment, the long chain PAM may be fragmented by these forces through exposure to the sun and tilling. To investigate the possibility that PAM of shorter chain length could be used as a C source, enrichment cultures were established in which various chain length PAMs and UV exposed PAM were added as the sole C source.

Aliquots of PAM stock solution (25 mL; 0.1%) were dispensed into 150 mm x 15 mm sterile plastic petri dishes and placed under a UV light source for 24 hours.

Size exclusion HPLC was used to identify changes in PAM chain length following UV treatment. The method used was a modification of that described by Leung et. al. (16). A Gilson HPLC was used, equipped with a Rheodyne injector (model 7125) and Progel-TSK GMP_{XL} column (Supelco, Bellefonte, PA). Samples (20 µL) were injected, eluded with 0.1 M Na_2SO_4 isocratically at 0.5 mL/min., and detected at 215 nm.

Enrichment cultures were established and incubated as described above except that UV treated PAM was supplied as the sole source of C or N.

In addition, mineral salts medium supplemented with reduced molecular weight PAM as the sole source of carbon was inoculated and serially tranfered as described above. PAM preparations of 3,000-4,000 MW, 12,000-15,000 MW and 200,000 MW were each added to media separately to yield a final concentration of 0.05%.

Amidase Determination

Amidase activity was determined for enrichment cultures able to use PAM as an N source. Cultures were grown in 25 mL of mineral salts medium supplemented with either NH_4NO_3 (0.024%), proprionamide (0.04%), or PAM (0.05%), as a source of N, and 0.05% mannitol and 0.05% acetate as a source of C. These concentrations yielded final C:N of approximately 6:1. The mineral salts medium used was identical to that described above except the $Na_2HPO_4 \cdot 7H_2O$ (17.74 g) was replaced with K_2HPO_4 (11.5 g), to reduce the concentration of Na^+, and allow detection of NH_4^+, the assay product using ion chromatography. The flasks were inoculated with 2 mL of enrichment culture (generated from PAM treated soil) in which PAM was utilized as the sole N source, and then incubated at 25° C for 48 h on a reciprocal shaker (200 rpm). Cells and culture supernatant were harvested via centrifugation at 10,000 g for 10 min at 4° C. The supernatant was collected and maintained on ice, while the cells were washed twice in 75 mM potassium phosphate buffer at pH 7.6. Cell-free extracts were obtained by sonication followed by centrifugation at 10,000 g for 10 min at 4° C.

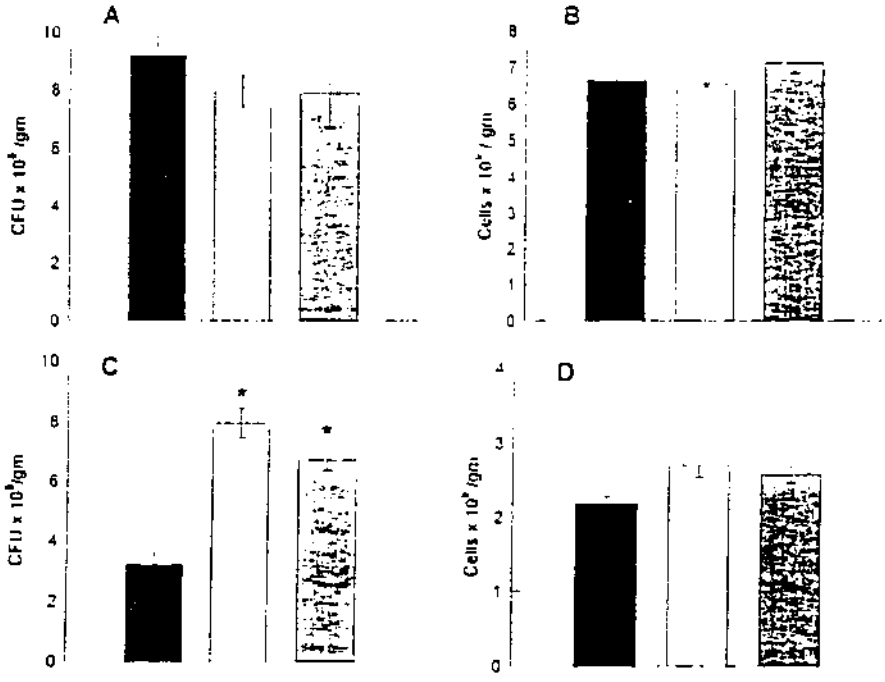
The cell free extract and the culture supernatant were pooled and used as enzyme source in the amidase assay procedure described by Friedrich and Mitrenga (6). The substrates used were formamide, proprionamide and PAM, at concentrations of 100 mM, 100 mM, and 0.025% respectively. Assay mixtures were established in triplicate for each amide and enzyme combination. The

assay mixtures were incubated at 30° C for 2 h, then acidified with 1 M HCl to pH 5.5 and maintained on ice. Amidase activity was immediately determined by the concentration of NH_4^+-N released from the test substrate. Ammonium concentration was determined by ion chromatography as describe above. Controls containing only buffer + enzyme, and buffer + amide were also analyzed for NH_4^+ in order to determine the amount of NH_4^+ present in the test mixtures that was specifically due to amidase activity.

Statistical Analysis

Statistical comparisons between field sample analyses were conducted using the one-way ANOVA, with a confidence interval of 0.05 ($p < 0.05$) (26). Comparison of culture amidase activity between the different N sources and amide substrates were analyzed using the Student's T-test, with a confidence interval of 0.05 ($p < 0.05$) (26).

Figure 1



*Microbial populations in PAM treated and untreated soils. Culturable heterotrophs (A) and Acridine Orange Direct Count (B) in soil planted to beans, and culturable heterotrophs (C) and Acridine Orange Direct Count (D) in soil planted to potatoes. Untreated soil ■, PAM treated soil □, oil-PAM emulsion (bean soil) and high PAM flow rate (potato soil). *Indicates significantly different from the control, p<0.05.*

Results — Enumeration of Bacteria in Soils

Heterotrophic plate count data revealed no significant differences between treated and untreated soil planted to beans (fig. 1A). However in the soil planted to potatoes, there was a significant increase in culturable heterotrophs in treated soil as compared to untreated soil (fig. 1C). The AODC data indicate that total bacterial numbers were not significantly altered in the PAM treated soil as compared to untreated samples in either crop type (fig.1B,D).

Inorganic Nitrogen Pool Determination

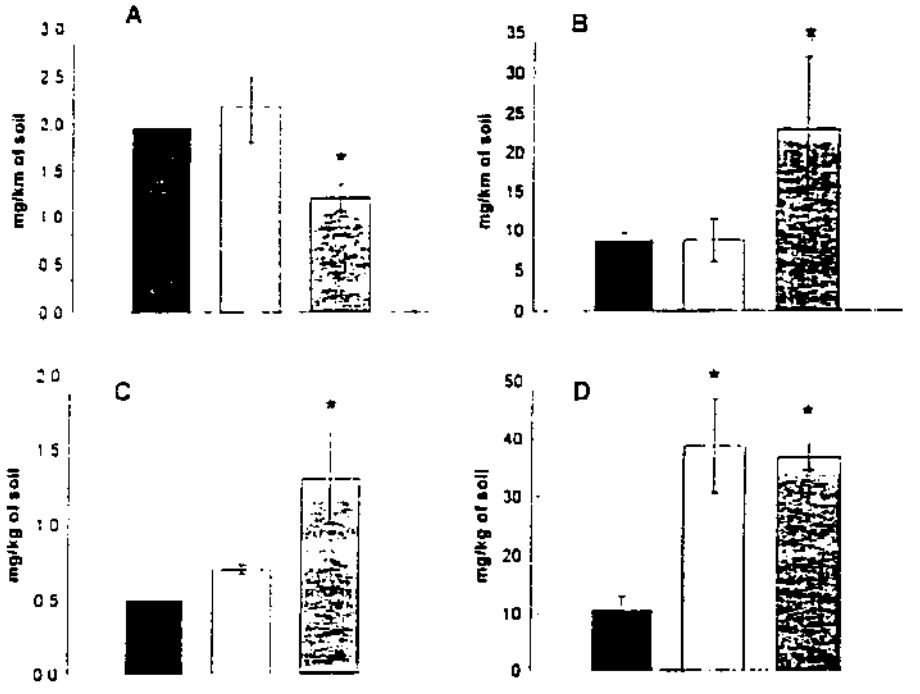
Inorganic N pools were significantly different between PAM treated and untreated samples only under specific circumstances. PAM treated potato field soil had an elevated level of KCl extractable NO_3^--N as compared to untreated samples (fig.2D). The impact on the KCl extractable soil NH_3-N appeared to depend on the PAM application flow rate (fig.2C). While the NH_3-N concentrations in untreated soil and soil treated with PAM at a normal flow rate were not significantly different, the soil collected from furrows which were subject to higher initial flow rates did exhibit a higher concentrations of KCl extractable NH_3-N. The NO_3^--N and NH_3-N concentrations in the PAM treated samples collected from the bean field were not significantly different from the concentrations in the untreated samples (fig.2A,B). There was, however, a significant difference in both NO_3^--N and NH_3-N concentrations in samples that had been treated using a PAM-mineral oil emulsion (fig.2A,B).

Enrichment Cultures and Growth Curves

To determine the extent to which PAM and its monomeric subunits (e.g. AA and AM) could be used as C and/or N source, enrichment cultures were established with PAM treated and untreated soil as inocula. Ability of organisms to grow utilizing the various C and N sources are shown in table 1. Enrichments were established which were able to use AM as a C and N source, acrylic acid as a C source, and PAM as a N source. No enrichments were obtained that were able to use PAM as a sole carbon source. There were no differences between treated and untreated inocula with respect to substrate utilization ability.

No substantial differences were observed between the growth of enriched populations from treated soils vs. untreated soils using PAM or AM as a N source (fig.3A,B) and for enrichments using AM as both N and C (fig.3E). For enrichments supplied with AM or AA as a sole C source, some differences between treated and untreated soil enrichments were observed (fig 3C,D). Enrichments generated from PAM treated soils utilizing AM as a C source displayed a enhanced growth response compared to enrichments from untreated soils. The opposite pattern was observed for enrichments utilizing AA as a C source

Figure 2



*KCl extractable inorganic N pools in PAM-treated and untreated soils. Ammonia-N (A) and nitrate-N (B) in soil planted to beans, and ammonia-N (C) and nitrate-N (D) in soil planted to potatoes. Untreated soil ■, PAM treated soil □, oil-PAM emulsion (bean soil) and high PAM flow rate (potato soil) . * Indicates significantly different from the control, p<0.05.*

Table 1. Enrichment culture growth using PAM treated and untreated soils as inocula (+ = growth, - = no growth).

C source	N source	treated soil culture	untreated soil culture
None	NH_4NO_3	-	-
PAM	NH_4NO_3	-	-
Acrylamide	NH_4NO_3	+	+
Acrylic Acid	NH_4NO_3	+	+
Glucose	None	-	-
Glucose	PAM	+	+
Glucose	Arylamide	+	+
Glucose	Acrylic Acid	-	-
None	None	-	-
PAM	PAM	-	-
Acrylamide	Acrylamide	+	+
Acrylic Acid	Acrylic Acid	-	-

Abiotic Release of NH_3/NH_4^+ in Culture

Levels of NH_3/NH_4^+ released abiotically during incubation of medium containing PAM indicate that negligible NH_3/NH_4^+ was released (0.10 ppm). A small amount of free NH_3/NH_4^+ (0.35 ppm) was present in the culture medium prior to incubation.

UV Exposed PAM and SEHPLC

Treatment with UV light effectively reduced the chain length as was evidenced by the production four species with longer retention times determined by SEHPLC (data not shown). No growth was observed in the enrichment cultures with carbon present as UV treated PAM or PAM of MW as low as 3,000-4,000 (data not shown).

Treatment of PAM with UV light did not significantly alter the capacity of the enrichments to utilize PAM as a source of N (fig.3F). Enrichments generated using UV treated PAM and those generated with high MW PAM exhibited similar growth responses.

Amidase Determination

The enrichment that utilized PAM as a N source produced amidase when cultured with PAM. This enrichment did not exhibit amidase activity when cultured with NH_4NO_3 (table 2). The amidase activity exhibited is apparently inducible and demonstrated a much broader substrate specificity than the amidase(s) induced in the presence of proprionamide. The PAM induced amidase(s) demonstrated more pronounced activity toward PAM as a substrate than toward the smaller amide substrates tested.

Discussion — Enumeration of Bacteria in Soils

The impact of PAM application appears to be site specific in the soils examined in this study. In the soil planted to beans, no significant impact was observed with respect to either the number of culturable heterotrophs or total bacteria (fig.1A,B). In the soil planted to potatoes, PAM treatment did correspond to an increase in culturable heterotrophs, while the bacterial AODC counts were unchanged (fig. 1C,D).

Nadler and Steinberger (17) examined culturable bacterial numbers in three soil types with two PAM preparations at differing concentrations and the results were variable. Steinberger et al.(23) determined the effect of PAM application on microbial biomass to be unpredictable when determined in several soil types using a range of PAM concentrations. Changes in soil microbial population

levels due to PAM application is likely dependent on many factors such as crop type, soil characteristics, moisture regime, nutrient status, and the type of PAM preparation applied. Any impact of PAM on microbial population levels may be obscured by other environmental parameters.

Inorganic Nitrogen Pool Determination

The effects of PAM application on inorganic N pools also appears to be site specific. In the soil samples planted to potatoes, an increase in both NO_3^--N or NH_3-N concentrations was observed in samples obtained from furrows receiving PAM treated water at a high initial flow rate (fig 2C,D). At the normal flow rate, only the NO_3^--N was elevated, indicating that the PAM treatment may have altered the amount of NO_3^- produced or utilized by soil biota. The observation that only the high flow PAM treatment resulted in elevated soil NH_3-N concentration may be due to a higher PAM dose in those furrows, resulting in a higher cumulative concentration at which an effect could be observed. It does not appear that the increase in NH_3-N observed was due to the flow rate itself because controls were run comparing high and normal flow untreated furrows and no difference was observed between them.

In the soil samples planted to beans, no difference was observed between untreated soil and PAM treated soil with regard to KCl extractable NO_3^--N or NH_3-N (fig 2A,B). The abundance of N in soil planted to legumes may have overwhelmed any small changes in NO_3^--N or NH_3-N resulting from PAM application.

It appears that PAM application to agricultural soils can alter inorganic N concentrations under certain conditions. This may be due to changes in N transforming microbial population levels or altered availability of N substrates in the soil environment. It has been previously demonstrated that application of acrylamide resulted in an increase in NO_3^- and NO_2^- in soil (2). A similar phenomenon may be occurring in PAM treated soils. It possible that the addition of PAM to agricultural soils may result in low level release of N into the soil, thus providing a slight fertilization effect.

Table 2. Total amidase activity of PAM utilizing bacterial consortium grown on different substrates.

N source/Amide in growth medium	Specific activity for given amidase substrate (units/mg protein)		
	Formamide	Propionamide	PAM
NH_4NO_3	0	0	0
Proprionamide	0	1.00±0.038	0.071±0.017
PAM	1.01±0.305	1.01±0.084	7.49±0.85

The use of the PAM-mineral oil emulsion as an irrigation water additive resulted in a significant decrease in NH_3-N and a significant increase in NO_3^--N when compared to untreated and PAM treated soil planted to beans(fig. 2A,B). Because the PAM preparation without mineral oil did not result in this effect, it appears that the mineral oil component may be responsible for the impact observed. The mineral oil may have been used as a carbon substrate by some soil organisms resulting in a shift in population composition that may in turn influence the inorganic N pools in the soil.

Enrichment Cultures

It appears that naturally occurring soil microorganisms are capable of utilizing PAM and the monomer acrylamide as a sole source of N. Enrichment cultures were established that could used the monomers that make up the PAM polymer (AM and AA) as a carbon source, but no enrichments were able to use PAM as a source of C (table 1).

The large size of the PAM molecule could certainly restrict its availability to bacterial cells. Because environmental factors such as UV and shear forces have been shown to reduce chain length (18, 24), smaller PAM molecules and UV treated PAM preparations were used in enrichment efforts to culture organisms capable of utilizing smaller versions of PAM as a sole source of C. No culturable organisms capable of utilizing these smaller forms of PAM were observed from PAM treated or untreated soils.

Bacterial species exist that are capable of utilizing acrylate oligomers as C source (11, 10), and Hayashi et al. (9) have reported that two groups of bacteria enriched from soil, when cultured together, are able to degrade polyacrylate molecules up to 3,000 MW. It is possible that soil organisms exist that are capable of utilizing and thus degrading PAM but are either unculturable or were unable to grow under the conditions used here.

In addition to abiotic forces (e.g. UV irradiation and shearing) that may cleave the large PAM molecule into smaller fragments that are more suitable for bacterial utilization, exoenzymes secreted by soil fungi and bacteria could potentially play a role in reducing chain length. No data supporting these theories of abiotic or biotic *in situ* chain length shortening are available due to technical difficulties associated with desorbing and assaying for PAM in soil systems. Alternatively, if the large PAM molecules remain intact in the soil, or are fragmented but still recalcitrant, then PAM may ultimately be converted into the humic fraction of the soil. A similar phenomenon has been described regarding incorporation of recalcitrant pesticide residues into humics by Alexander(1).

Commercially available PAM is restricted by law to contain no more than 0.05% acrylamide monomer (4). The acrylamide monomer is a potent neurotoxin (19). Our data indicate that indigenous microbial populations in both PAM treated and untreated soil are capable of rapidly degrading low concentrations of acrylamide(table 1), similar to that present in the PAM preparations. This conclusion is supported by the findings of Shanker et. al. (22) who reported

complete degradation of acrylamide by soil microbes within five days of applying 500 mg/kg of AM to garden soil. Lande et. al. (13) also reported half-life values of acrylamide in soil ranging from 18 to 45 h when 25 mg/kg was applied to various soil types. An estimate of the amount of acrylamide applied to a given furrow per irrigation event using 10 ppm PAM is approximately 1.2×10^{-3} mg/kg assuming 6 ins. of penetration.

Growth Curves of Enrichments

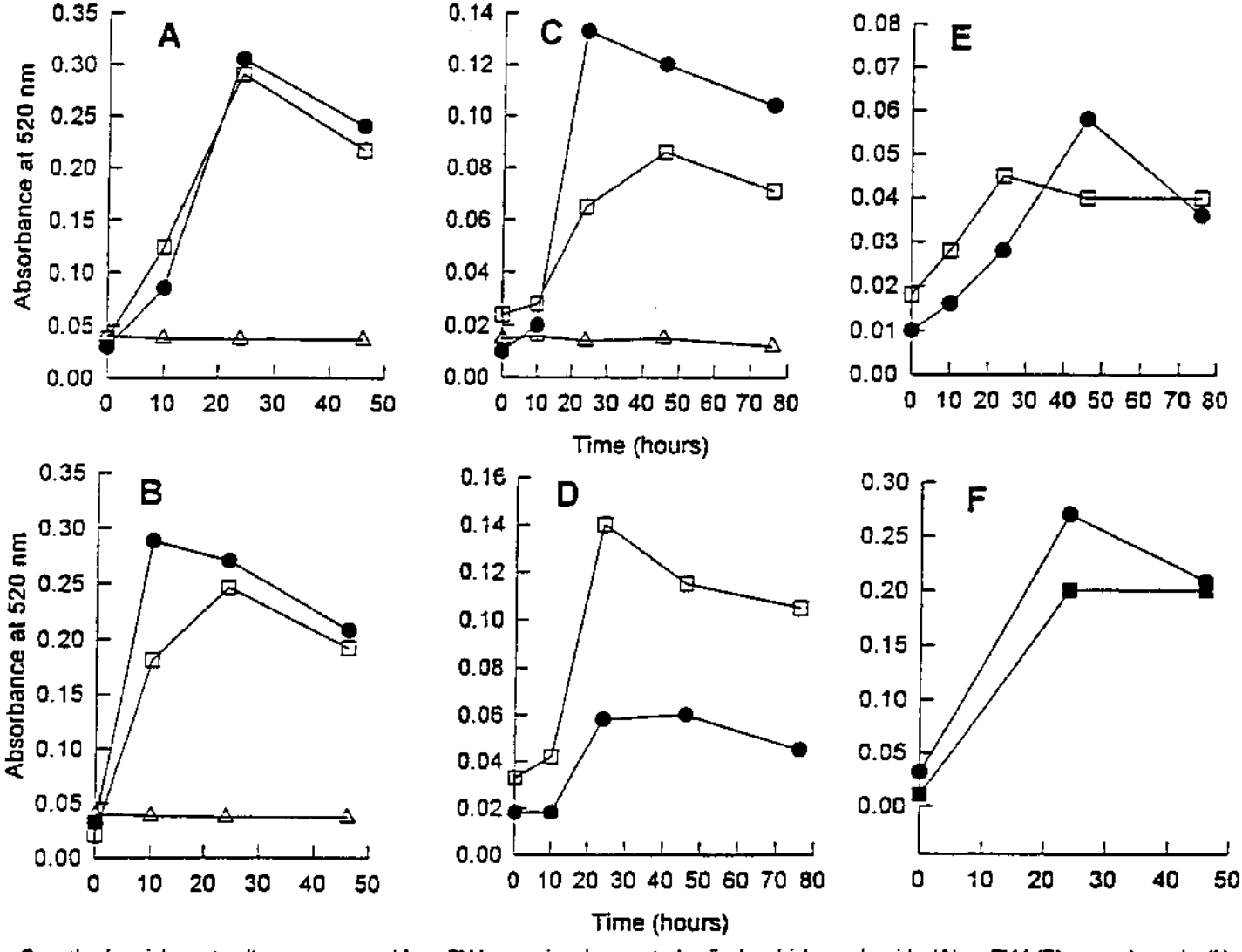
Growth curve data were collected to compare growth response patterns of enrichments derived from PAM treated and untreated soils to ascertain whether populations in those soils differed due to PAM application. Enrichments able to use PAM as N, AM as N, and AM as C and N demonstrated similar growth responses regardless of origin of soil inoculum (fig.3A,B,E). These results imply that similar populations exist in the soils regardless of PAM treatment. Enrichments generated from PAM treated soil that used AM as a C source demonstrated an elevated growth response as compared to that of the untreated soil enrichment (fig.3C). This may reflect the existence of a selective pressure or advantage in soil treated with the PAM preparation in which contaminating AM is present. However, in enrichments that used AA as a source of C, untreated soils demonstrated an elevated growth response (fig.3D). This result contrasts with the AM enrichment culture growth curve data and with the proposed mechanism of bacterial utilization of AM (22) in which AM is converted into AA via amidase action, followed by rapid mineralization of AA.

To investigate whether use of UV exposed PAM during the enrichment process would select a different population, UV exposed PAM and unexposed high MW PAM were used as N sources in the generation of enrichment cultures. The growth curves of those enrichments imply no difference in the enrichment populations based on growth response patterns (fig.3F).

Abiotic release of NH3/NH4+ in culture

Ability of enrichments to use PAM as a source of N was presumably due

Figure 3



Growth of enrichment cultures generated from PAM treated and untreated soils, in which acrylamide (A) or PAM (B) were the sole JN source, acrylamide (C) or acrylic acid (D) were the sole C source, acrylamide was the sole C and N source (E). Inocula originating from PAM treated soil ●, untreated soil □ and controls in which the mdium lacked a N source ▲. Enrichment cultures generated from PAM treated soil, in which UV treated PAM ● and untreated high MW PAM ■ were the soil N source (F).

to hydrolysis of the amide bond resulting in the release of NH_3/NH_4^+, either through abiotic hydrolysis or enzymatic action. The data indicate that a minute quantity of NH_3/NH_4^+ was released during incubation (0.10 ppm over 72 hours), in addition to a very small amount present as an initial contaminant (0.35 ppm). The sub-ppm concentration of NH_3/NH_4^+ present is inadequate to support observable cell growth. Consequently, abiotic hydrolysis does not appear to play a substantial role in the utilization of PAM as a N source by the enrichments.

Amidase Determination

The utilization of amides as a source of N has been reported in a variety of organisms including bacteria (5). Amidase (EC 3.5.1.4) enzyme activity is one of the mechanisms by which amides can be used as a source of N via the following reaction:

$$RCONH_2 + H_2O \rightarrow RCOOH + NH_3$$

This type of enzyme activity has been associated with utilization of the monomer AM by soil microbes (22), and this mechanism was investigated as a possible pathway by which bacteria in the enrichments were gaining N benefit from PAM.

Enrichment cultures that were able to utilize PAM as a sole N source did exhibit amidase activity (table 2). The enrichments which utilized PAM as a sole source of N probably were not able to transport the intact PAM molecule into the cell given its large size (approximately 0.2 µm). It is likely that an initial transformation must take place external to the cell before the cell can gain benefit from the PAM molecule. Extracellular amidase catalyzed hydrolysis may be responsible for this transformation. This hydrolysis would result in the release of NH_3/NH_4^+, which could be readily transported into the cell and used as a source of N.

The amidase (or amidase suite) produced by the enrichment culture is inducible and shows activity toward several aliphatic amides. The amidase, however, appears to have an increased activity when reacting with the large PAM substrate. A different amidase or amidase suite appears to be induced in the presence of propionamide which shows only slight activity toward the large PAM molecule.

It is possible that a similar phenomenon, the release of extracellular amidase by microbes to gain N benefit from the PAM, may occur in the soil environment. If PAM can be used by some microbes as a source of N via amidase release of N into the soil environment, it is not difficult to imagine that inorganic N concentrations in the soil could be affected as was observed in the field soil planted to potatoes (fig.2A,B).

Summary

These results indicate that PAM treatment of agricultural soils may impact soil microbial ecology and nutrient cycling patterns, and these effects appear to be site specific. In addition, PAM may potentially be used by soil bacteria as a source of N resulting in at least a partial degradation of the molecule.

Acknowledgements

We would like to thank Drs. Bob Sojka and Rick Lentz for their assistance.

References

1. Alexander, M. 1994. Biodegradation and Bioremediation. Academic Press Inc. San Diego, CA.

2. Abdelmagid, H.M. and M.A. Tabatabai. 1982. Decomposition of acrylamide in soils. J. Environ. Qual. 11:701-704.

2. Azzam, R., O.A. El-Hady, A.A. Lofty, and M. Hegela. 1983. Sand-RAPG combination simulating fertile clayey soils. Parts I to IV. Internat. Atomic Energy Agency. SM-267/15:321-349.

4. Barvenik, F. 1994. Polyacrylamide characteristics related to soil applications. Soil Sci. 158:235-243.

5. Clarke, P. 1980. The utilization of amides by microorganisms. In J.W. Payne [ed.] Microorganisms and Nitrogen Sources. J.Wiley & Sons Ltd., New York, NY.

6. Friedrich, C. and G. Mitrenga. 1981. Utilization of aliphatic amides and formation of two different amidases by Alcaligenes eutrophus. J. Gen. Microbiol. 125:367-374.

7. Grula, M.M. and M. Huang. 1981. Interactions of polyacrylamides with certain soil pseudomonads. Dev. Ind. Microbiol. 22:451-457.

8. Grula, M.M., M. Huang, and G. Sewell. 1994. Interactions of certain polyacrylamides with soil bacteria. Soil Sci. 158:291-300.

9. Hayashi, T., H. Nishimura, K. Sakano, and Y. Tani. 1994. Microbial Degradation of Poly (sodium acrylate). Biosci. Biotech. Biochem. 58:444-446.

10. Hayashi, T., M. Mukouyama, K. Sakano, and Y. Tani. 1993. Degradation of sodium acrylate oligomer by an Arthrobacter sp. Appl. Environ. Microbiol. 59:1555-1559.

11. Kawai, F. 1993. Bacterial degradation of acrylic oligomers and polymers. Appl. Microbiol. Biotechnol. 39:382-385.

12. Keeney, D. and D. Nelson 1982. Nitrogen - Inorganic Forms. In A. Page, R. Miller, and D. Keeney [eds.] Methods of Soil Analysis, part 2. Agronomy 9:643-698. 2nd Ed. Am. Soc. Agron., Inc., Madison, WI.

13. Lande, S.S., S.J. Bosch, and P.H. Howard. 1979. Degradation and leaching of acrylamide in soil. J. Environ. Qual. 8:133-137.

14. Lentz, R.D., I. Shainberg, R.E. Sojka, and D.L. Carter. 1992. Preventing Irrigation Furrow Erosion with Small Applications of Polymers. Soil Sci. Soc. Am. J. 56:1926-1932.

15. Lentz, R.D., and R.E. Sojka. 1994. Field results using polyacrylamide to manage furrow erosion and infiltration. Soil Sci. Soc. Am. J. 158:274-282.

16. Leung, R., R. Pandey, and B. Das. 1987. Determination of polyacrylamides in coal washery effluents by ultrafiltration/size-exclusion chromatography-ultraviolet detection techniques. Environ. Sci. Technol. 21:476-481.

17. Nadler, A. and Y. Steinberger. 1993. Trends in structure, plant growth, and microorganism interrelations in the soil. Soil Sci. 155:114-122.

18.1 Randby, B. 1993. Basic reactions in the photodegradation of some important polymers. J.M.S.-Pure Appl. Chem. A30:583-594.

19. Reviews of Environmental Contamination and Toxicology. 1988. Acrylamide. 107:1-12.

20. Schmidt, E. and E. Paul 1982. Microscopic Methods for Soil Microorganisms. In A. Page, R. Miller, and D. Keeney [eds.] Methods of Soil Analysis, part 2. Agronomy 9:803-813. 2nd Ed. Am. Soc. Agron., Inc., Madison, WI.

21. Senft, D. 1993. Erosion takes a Powder. Agricultural Res. 41:16-17.

22. Shanker, R., C. Ramakrishna, and R.K. Seth. 1990. Microbial degradation of acrylamide monomer. Arch. Microbiol. 154:192-198.

23. Steinberger, Y., S. Sarig, A. Nadler, and G. Barnes. 1993. The Effect of synthetic soil conditioners on microbial biomass. Arid Soil Res. Rehab.7:303-306.

24. Tolstikh, L.I., N.I. Akimov, I.A. Golubeva, and I.A. Shvetsov. 1992. Degradation and stabilization of polyacrylamide in polymer flooding conditions. Intern. J. Polymeric Mater. 17:177-193.

25. Wollum, A. II. 1982. Cultural Methods for Soil Microorganisms. In A. Page, R. Miller, and D. Keeney [eds.] Methods of Soil Analysis, part 2. Agronomy 9:781-802. 2nd Ed. Am. Soc. Agron., Inc., Madison, WI.

26. Zar, J. 1996. Biostatistical Analysis. 3rd Ed. Prentice Hall Inc. Upper Saddle River, New Jersey.

About the authors

Jeanine L. Kay-Shoemake and Mary E. Watwood — Dept. of Biological Sciences, Campus Box 8007, Idaho State University, Pocatello, ID. FAX:(208)235-4570